Application Note 10479

SPME-GC-MS/MS for Identification and Quantification of Migration Contaminants in Paperboard Food Packaging

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Goal
To accurately determine presence of chemical migrants from paperboard packaging to food products using solid phase microextraction (SPME) followed by gas chromatography-triple quadrupole mass spectrometry.

Introduction
Packaging of foods is a key activity of the food industry. It helps protect the food from damage while keeping it fresh and free from microbial degradation. However, the materials used in food packaging can also cause degradation of the packaged food. Inappropriately packaged food may develop an unpleasant odor or off-flavor. It is important for food producers to ensure that packaging does not contribute to off-flavors or other toxic compounds that might be harmful to consumers.

Packaging may consist of several different materials, including plastics, paper, metals, and glass. The use of packaging materials for food is regulated all over the world. The European Union (EU) published the first regulation concerning packaging materials in 2003 in EU Framework Regulation EC 1935/2004. A number of other regulations followed; the most recent being Regulation EU/202/2014 in 2014, which focuses on plastic materials and articles intended to come into contact with food.

Packaging legislation stipulates the maximum level of chemicals that can be transferred from packaging material to food, chemicals that may not be used for production of food packaging material at all, and provides limits for compounds that can be present either in food or in the material itself.

To fulfill the legislation requirements and ensure food safety, it is necessary to monitor both food samples and composition of packaging materials. Food producers require screening and quantitative analytical methods for the express purpose of monitoring compounds of interest in packaging material.

In the past 20 years, many scientific papers have been published on packaging migrants, offering appropriate solutions for their monitoring. Different sample preparation procedures, analytical techniques, and detectors have been employed. In general, there are two main approaches. The first is to analyze the material itself in its final format, assuming that 100% of each compound will be transferred to the food. The second approach requires a migration study, with simulated conditions for the packaged food product. During the study, a simulated food product is placed in contact with the packaging material for a precisely defined period of time, under specially defined conditions, and subsequently analyzed.

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In this application note, the first approach was followed in developing and validating a GC-MS/MS method for determination of packaging migrants in paperboard. Paperboard can be produced from virgin paper, recycled paper, or a mixture of both. Recycled paperboard is more likely to contain a wide range of dangerous contaminants derived from the degradation of paperboard components, including printing inks, coatings, and adhesives. However, virgin material must also be monitored for unwanted compounds produced during the manufacturing process.

The technique used for this analysis includes solid phase microextraction (SPME) coupled to gas chromatography-triple quadrupole mass spectrometry (GC-MS/MS), enabling detection of volatile and semi-volatile sample components. This technique provides a major advantage because volatile compounds are the most likely to migrate from packaging to food.

SPME was investigated in 1990[1] and has been used in a wide range of applications for determination of volatiles in different food matrices, environmental samples, and packaging materials. This unique technique gained popularity due to the benefits it offers. SPME avoids extensive use of organic solvents by combining extraction and pre-concentration in a single step. Additionally, it allows for a significant reduction in sample handling and time-consuming sample preparation. Automated SPME is an effective method, well-suited to routine analysis.

For this study, automated SPME was applied to quantification of 12 representative possible migrants (phthalates, photoinitiators, phenols, and off-flavors) in paperboard.

Experimental Conditions

These experiments use a Thermo Scientific™ TSQ™ 8000 GC-MS/MS system, including a Thermo Scientific™ TRACE™ 1310 gas chromatograph equipped with a Thermo Scientific™ TriPlus™ RSH autosampler and SPME module (SPME NL: 50.5 mm). The column used for GC separation is a Thermo Scientific™ TraceGOLD™ TG-5SilMS, 30 m × 0.25 mm × 0.25 µm (P/N 10177894). Data acquisition for quantification and confirmation is performed in the timed-selected reaction monitoring (timed-SRM) mode using Thermo Scientific™ TraceFinder™ 3.2 software.

Sample Preparation

Cut the paperboard sample into small pieces (2 mm × 2 mm) and weigh 1 g into a 20 mL headspace vial. To the sample, add 8 mL of 13% CH_3OH in water. Close the vial and place into the autosampler tray. Once the vial is positioned in the tray, begin the SPME process, followed by GC-MS/MS, as shown in Figure 1.

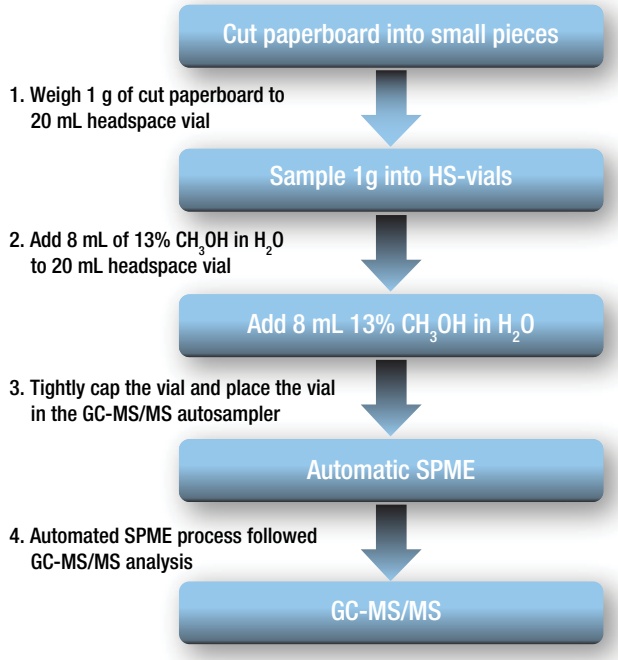

Figure 1. Schematic of method.

Automated SPME Analysis

The automated SPME procedure includes transferring the sample vial from the autosampler tray to a heated chamber to enable, extraction, adsorption of the analytes by the fiber and thermal desorption of the analytes from the fiber into the injector. The fiber is then conditioned in a fiber conditioning station in preparation for the next sample analysis, and the vial is transferred back to the autosampler.

SPME fiber:	100 µm PDMS (polydimethylsiloxane)
Incubation time:	0 min
Extraction time and temperature:	45 min at 65°C
Desorption time and temperature:	7 min at 270°C
Conditioning fiber:	20 min at 250°C
Swirling the vial:	constant

Calibration Standards

The calibration standards, listed below, were purchased from Sigma-Aldrich.

- 1-Hexanol
- 1-Methoxy-2-propanol
- 2,4-Di-tert-butylphenol
- 2-Ethyl-1-hexanol
- Allyl benzoate
- Benzaldehyde
- Benzophenone
- Di(propylene glycol) methyl ether
- Dimethyl phthalate (DMP)
- Ethyl benzoate
- Hexanal
- 2,4,6-Trichloroanisole

Spiking Standard Solution

Spiking standard solution was prepared by diluting individual stock solutions of seven analytes (1-hexanol, 1-methoxy-2-propanol, 2-ethyl-1-hexanol, benzaldehyde, di(propylene glycol) methyl ether, DMP and hexanal) and working standard solution in water. The analytes were divided into four groups with different concentrations (Table 1). The spiking standard solution was prepared fresh before each use.

RECOMMENDED CONDITIONS	
TRACE 1310 GC	
Liner:	SPME Liner 0.75 mm × 6.35 mm × 78.5 mm Restek P/N 21111-214.5
Carrier Gas:	He 1.2 mL/min
Column Type:	Thermo Scientific™ TraceGOLD™ TG-5SilMS, 30 m × 0.25 mm × 0.25 µm (P/N 10177894)
Column Oven:	40 °C, hold for 1 min. Ramp at 10 °C/min to 160 °C. Ramp at 12 °C/min to 200 °C. Ramp at 17 °C/min to 300 °C. Hold for 5 min
Instant Connect SSL Injector	
Inlet Temperature:	270 °C
Mode:	S/SL splitless, 5 min; split flow 50 mL/min
Run Time:	27.5 min
TSQ 8000 MS	
Transfer Line (°C):	250
Ionization Type:	EI
Ion Source (°C):	250
Electron Energy (eV):	70
Acquisition Mode:	timed-SRM (Table 2)
Resolution:	Q1 normal (0.7 Da)
Collision Gas:	Argon
Minimum Baseline Peak Width (s):	3
Desired Scans per Peak:	15

Table 1. Preparation of spiking standard solution.

Compounds	Group	Concentration of Stock Standard /Working Standard (µg/mL)	V (mL) to 10 mL Flask from Stock Standard / Working Standard	Concentration of Spiking Standard Solution (µg/mL)
1-Hexanol	3	1000	0.1	10
1-Methoxy-2-propanol	2	1000	0.01	1
2,4-Di-tert-butylphenol*	1	10	0.1	0.1
2-Ethyl-1-hexanol	2	1000	0.01	1
Allyl benzoate*	1	10	0.1	0.1
Benzaldehyde	2	1000	0.01	1
Benzophenone*	1	10	0.1	0.1
Di(propylene glycol) methyl ether	4	1000	1	100
DMP	2	1000	0.01	1
Ethyl benzoate*	1	10	0.1	0.1
Hexanal	4	1000	1	100
2,4,6-Trichloroanisole*	1	10	0.1	0.1

*analytes from Group 1 are mixed together to create the working calibration standard solution

Table 2. GC-MS/MS parameters for selected reaction monitoring of analytes.

Name	RT (min)	Quantifier Ion			Qualifier Ion		
		Precursor Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (eV)	Precursor mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (eV)
1-Hexanol	4.68	56.1	41.0	10	69.1	41.1	10
1-Methoxy-2-propanol	7.22	69.9	55.1	10	56.9	41.0	5
2,4-Di-tert-butylphenol	14.01	206.3	191.2	10	191.1	57.1	13
2-Ethyl-1-hexanol	7.23	56.9	41.1	5	82.9	41.1	15
Allyl benzoate	10.74	105.0	77.1	12	105.0	50.9	27
Benzaldehyde	6.19	106.1	105.0	10	106.1	77.0	20
Benzophenone	15.47	105.1	77.1	10	181.9	105.1	10
Di(propylene glycol) methyl ether	6.99	104.2	58.9	10	59.0	43.0	20
DMP	13.31	163.0	77.1	20	163.0	133.1	10
Ethyl benzoate	9.47	105.0	77.0	10	105.0	50.9	25
Hexanal	3.65	56.5	40.9	5	56.5	31.1	20
2,4,6-Trichloroanisole	11.74	194.9	166.9	13	209.7	194.9	10

Calculations

Identification

Identification of the packaging migrants was based upon the presence of transition ions (quantifier and qualifier) at the retention times (+/- 2.5%) corresponding to known standards. In timed-SRM mode, the measured peak area ratios for qualifier to quantifier ion should be in close agreement (according Commission Decision 2002/657/EC[2]) with those of the standards, as shown in Table 2. The quantifier and qualifier ions were selected, based on intensity, from among the product ions produced by the fragmentation of the selected parent ion.

Quantification

Matched calibration was used for the quantification matrix. A calibration curve was plotted as the peak area is a linear function of the concentration of the analyte. The analyte concentration in the sample was determined using the equation:

$$c_a = \left(\frac{A_a - b}{a}\right)$$

c_a = analyte concentration in µg/kg
A_a = peak area of the analyte
b = y-intercept
a = slope of the calibration curve

Method Performance

The method was validated in-house using the criteria specified in European Commission Decision 2002/675/EC[2] as a guideline, as no guidelines specific to packaging migrants currently exist. Validation parameters were determined by spiking virgin paperboard at three different levels. The measured parameters were specificity, linear range, repeatability, accuracy, limit of detection (LOD), and limit of quantification (LOQ).

Specificity

Using SRM, specificity was confirmed based on the presence of the transition ions (quantifier and qualifier) at the correct retention times, corresponding to those of the respective analytes. The measured peak area ratios of qualifier/quantifier are in the range defined in the Commission Decision 2002/657/EC[2] compared to the standards (Table 3).

Linearity and calibration curve

Calibration curves were created from eight matrix-matched calibration standards, which were prepared and injected in duplicate for each batch. Correlation coefficients and linear ranges are shown in the Table 3.

Table 3. Ion ratios (Qual/Quan) in matrix and in standard mixture (the agreement between ion ratios should be in the permitted tolerance, which is defined in the Commission Decision 2002/657/EC).

Compound	R^2	Linear range (µg/kg)	Ion ratio - matrix (%)	Ion ratio - solvent (%)
1-Hexanol	0.9928	0–6000	54.21	57.26
1-Methoxy-2-propanol	0.9925	0–600	93.77	95.61
2,4-Di-tert-butylphenol	0.9915	0–60	99.9	106.4
2-Ethyl-1-hexanol	0.9947	0–600	17.44	14.16
Allyl benzoate	0.9918	0–60	31.84	32.97
Benzaldehyde	0.9937	0–600	62.3	60.8
Benzophenone	0.9931	0–60	19.7	38.2
Di(propylene glycol) methyl ether	0.9940	0–60000	33.47	27.2
DMP	0.9930	0–600	42	42.8
Ethyl benzoate	0.9920	0–60	30.64	30.8
Hexanal	0.9909	0–60000	75.6	77.2
2,4,6-Trichloroanisole	0.9902	0–60	47.37	47.5

Table 4. Results of method precision (expressed as relative standard deviation - RSD) and accuracy (expressed as recovery) at three different spike levels (six replicates).

Compound	Spiking Levels (µg/kg)			RSD (%)			Recovery (%)		
	I	II	III	I	II	III	I	II	III
1-Hexanol	750	2000	4000	8	14	2	76	94	100
1-Methoxy-2-propanol	75	200	400	17	5	1	107	115	107
2,4-Di-tert-butylphenol	7.5	20	40	11	13	13	87	86	83
2-Ethyl-1-hexanol	75	200	400	17	4	1	106	114	106
Allyl benzoate	7.5	20	40	14	14	5	87	98	95
Benzaldehyde	75	200	400	14	5	1	97	112	108
Benzophenone	7.5	20	40	8	6	2	91	101	96
Di(propylene glycol) methyl ether	7500	20000	40000	22	10	22	76	71	76
DMP	75	200	400	9	8	3	96	104	103
Ethyl benzoate	7.5	20	40	14	10	3	88	99	97
Hexanal	7500	20000	40000	21	10	6	98	119	117
2,4,6-Trichloroanisole	7.5	20	40	17	22	21	103	91	86

Table 5. Limits of detection and quantification of the method (LOD and LOQ) and method intermediate precision expressed as RSD (%)—at one level—three sets measured with six replicates in three days.

Compound	LOD (µg/kg)	LOQ (µg/kg)	Intermediate precision at Level II (%)		
			Day 1	Day 2	Day 3
1-Hexanol	100	300	8	16	12
1-Methoxy-2-propanol	20	60	17	12	1
2,4-Di-tert-butylphenol	0.3	1	11	5	16
2-Ethyl-1-hexanol	20	50	17	10	1
Allyl benzoate	0.3	1	14	8	6
Benzaldehyde	2	5	14	9	3
Benzophenone	16	50	8	7	3
Di(propylene glycol) methyl ether	2500	7500	22	14	15
DMP	8	20	9	7	9
Ethyl benzoate	1.5	5	14	9	3
Hexanal	35	100	21	14	8
2,4,6-Trichloroanisole	0.03	0.1	17	7	21

Precision

Precision (repeatability) of the method was determined using independently spiked virgin paperboard samples at three different levels. A set of three concentrations with six repetitions each was analyzed in the same day. For the determination of the intermediate precision, two other sets at a single concentration, with six repetitions were measured over the next two days. The results are shown in Tables 4 and 5. Repeatability was within normal deviations except for the less volatile compounds, such as 2,4,6-Trichloroanisole.

Accuracy

Method accuracy was determined using independently spiked virgin paperboard samples at three different levels. Accuracy was evaluated by comparing found values with standard addition in spikes. Recovery values are shown in Table 4.

Limits of detection (LOD) and quantification (LOQ)

Limits of detection and quantification are estimated following the IUPAC approach, which consists of analyzing the blank sample to establish noise levels and then estimating LODs and LOQs for signal/noise at 3 and 10, respectively. The values are shown in the Table 5.

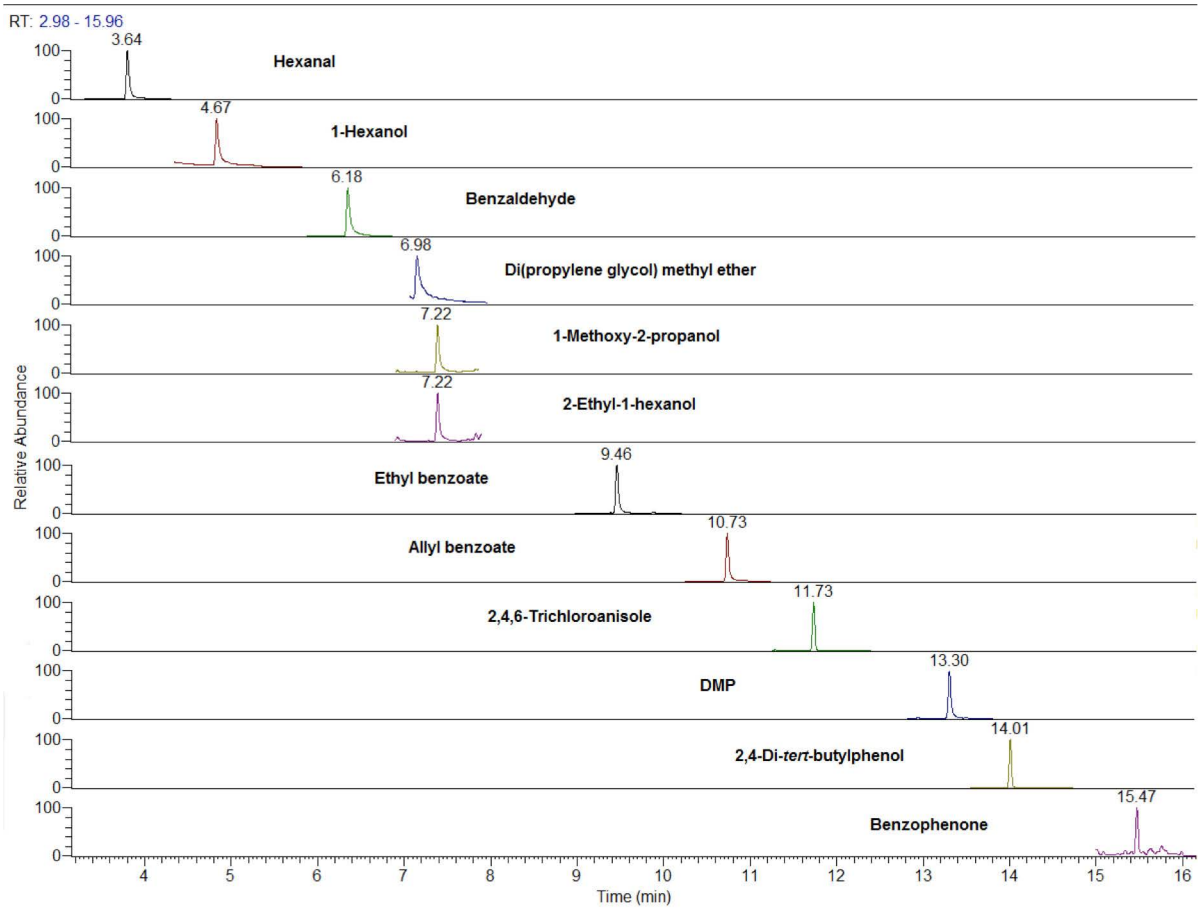

Figure 2. Chromatogram of spiked paperboard with 12 packaging migrants (c = 0.024–30 mg/kg).

Conclusion

The reported in-house validated method enables determination and quantification of 12 possible migrants from paperboard packaging. This fully automated SPME method can increase laboratory throughput. The results obtained from in-house validation, according to the IUPAC/AOAC harmonized protocol, confirmed that this method is suitable for monitoring the content of unwanted contaminants in paperboard intended for with contact with food.

- The method is fully automated due to the use of automated SPME
- Due to the automation, the method is very fast, robust, and saves manpower
- Use of the TSQ 8000 mass spectrometer offers the advantages of high sensitivity and easy routine maintenance

References

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2. Commission Decision 2002/657/EC. *Off. J. Eur. Commun*. **2002**, L221/8.

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